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On next page

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Abstract. We tested the hypothesis that inflammation results in detectable alteration of body odors. Using an animal model, we trained biosensor mice to distinguish between urine odors from lipopolysaccharide-treated and control mice. Lipopolysaccharide (LPS) is a general elicitor of inflammation. Trained biosensors could distinguish between the odors of LPS-treated and control mouse urine. Chemical analyses further demonstrated that LPS-induced inflammation results in alteration of urine volatiles. Importantly, urine samples collected many days following LPS-administration were discriminable. Thus, odor differences were not produced by acute effects of LPS-treatment (e.g. dehydration); nor were they likely related to changes in cytokines which usuallyoccur within hours of LPS exposure.

We similarly demonstrated odor alteration due to treatment with LPS in humans. Urine samples collected from humans receiving a small dose of LPS (or control) were subjected to discrimination tasks by a human sensory panel as well as chemical analyses. Both assays suggested that treatment with LPS results in a detectable alteration of urine volatiles.

From these experiments, we conclude that LPS-induced inflammation alters urine in both mice and humans. Furthermore, these changes can be detected by olfaction. Future work will focus on patterns of volatiles associated with traumatic brain injury (TBI) and determine if there are similarities and differences between inflammation caused by LPS and TBI.

Table of Contents

1. INTRODUCTION:

Chemical signals are the primary form of social communication for many species (Brennan & Kendrick 2006, Johnston 2003, Kelliher 2007). Although most research has been devoted to communication of social messages such as sex, age, and individual identity, volatile odorants may also communicate information about an animal's health status (Kavaliers *et al.* 2005, Moser & McCulloch 2010, Penn & Potts 1998). The mechanisms underlying changes in body odor caused by disease are poorly understood and the specificity of odor changes to a specific disease has rarely been explored. Immune function represents an interesting pathway for diseases to alter body odor (Beauchamp & Yamazaki 2005, Beauchamp *et al.* 1985, Brown & Eklund 1994). Based on this reasoning, we propose that chemical signals, acting through small volatile molecules (that is odorants), can be used to monitor immune activation and inflammation in humans and other animals. The ultimate goal of this work is to develop biosensors and chemometric approaches that can be successfully used to 'eavesdrop' on metabolic processes associated with inflammatory processes.

2. KEYWORDS:

Biosensor; Body Odor; Human; Inflammation; Lipopolysaccharide (LPS); Mouse Model; Traumatic Brain Injury (TBI); Volatiles

3. OVERALL PROJECT SUMMARY:

Approval to amend the milestone deadlines was received on 05-Apr-13. Please note that no actual milestones (nor proposed methods) were changed. The following schedule reflects those approved changes:

Milestone 1 (Experiment 2) – Y1Q3, 15-Jul-13
Milestone 4 (Experiments 5, 6) – Y1Q4, 15-Oct-13
Milestone 2 (Experiment 1) – Y2Q1, 15-Jan-14
Milestone 3 (Experiments 3, 4) – Y2Q2, 15-Apr-14

To date, Milestones 1 and 4 are complete; Milestones 2 and 3 are on-schedule.

Milestone 1. Demonstration that lipopolysaccharide (LPS) induces odor changes in the mouse model using both animal biosensors and chemometric analyses was completed in Y1Q4. These results demonstrated that urine collected following treatment with LPS can be discriminated from urine collected from control subjects (treated with phosphate-buffered saline, PBS) on the basis of odor. Importantly, urine samples were collected 11 to 14 days post-treatment. Thus, the source of these odors was not related to acute symptoms of inflammation (such as dehydration) which lapse earlier than the urine collection date.

In rewarded training trials, mouse biosensors achieved 93% accuracy in their discrimination of LPS from PBS urine samples (Experiment 2). The onset of unrewarded

extinction trials resulted in a 6% loss in accuracy. These two phases of training utilize LPS and PBS urine samples from the same donors.

Unrewarded generalization trials were conducted with LPS and PBS urine collected from novel donors. Accuracy significantly greater than 50% indicates that a biosensor is discriminating LPS from PBS, not simply discriminating between unique donor odortypes used during training. Each of the six biosensors performed significantly better than chance in the LPS versus PBS generalization trials (Figure 1). Cumulatively, biosensors responded with 75% accuracy.

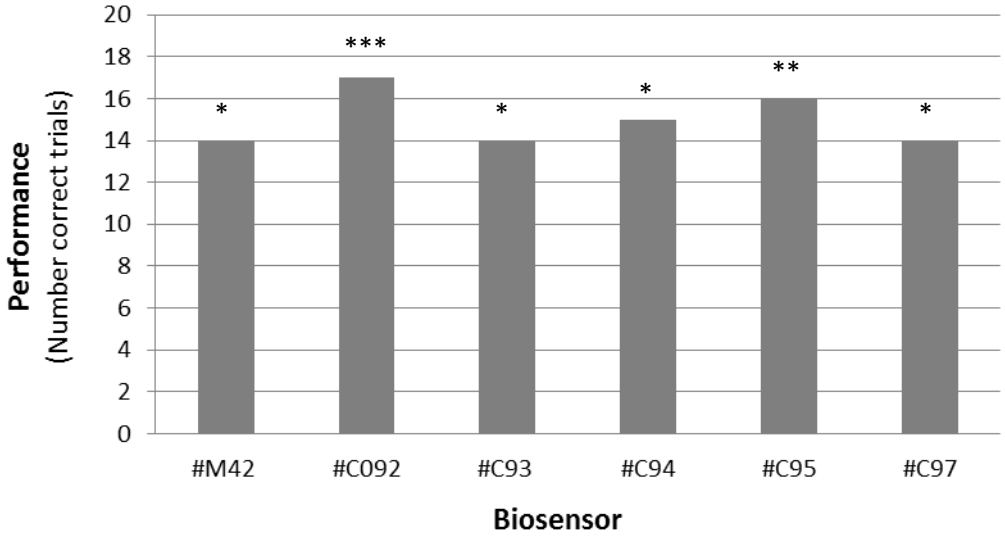

Figure 1. Mouse biosensor performance in 20 generalization trials. Trials were divided by urine collection day (Days 11-14) into 4 sessions, each of which included 5 generalization trials with urine from a single collection day.
(* $p<0.05$; ** $p<0.01$; *** $p<0.001$)

Chromatographic data from headspace gas chromatographic analyses of mouse urines also indicates that treatment with LPS induced changes in volatile profiles in the mouse model, consistent with the mouse biosensor results reported above. Urine samples were collected from mice treated with either a single LPS or single control (PBS) injection and the chromatographic data from a total 514 urine samples (including multiple collections from each donor across multiple collection days) were subjected to linear discriminant analysis.

Using only three peaks (chromatographic response from an individual volatile chemical), 18 of 20 samples collected from mice receiving LPS and 18 of 20 samples collected from mice receiving control were correctly categorized. The error estimate upon cross-validation was 10.0%.

Milestone 4. Characterization of changes in human body odor in response to treatment with LPS by human sensory panel and by chemometric analyses was completed in Y1Q4. The results of Experiment 5 ("Psychometric analyses of odor samples from LPS-treated human donors") suggested that LPS injection induces changes in the volatiles emitted by human urine odor, which are detectable to humans and differ from the changes that occur naturally over the course of a day. Results of Experiment 6 ("Chemometric analyses of odor samples from LPS-treated human donors") strongly supported these suggestive sensory results.

Human biosensor evaluation of human urine suggests that lipopolysaccharide (LPS) injection induces changes in the urine volatiles which differ from naturally occurring changes in odor over the course of a day. Twenty-one human biosensors each performed two within-donor, three-alternative, forced-choice (3AFC) discrimination tasks. One task included urine samples from LPS-treated urine donors and the other included samples from urine donors treated with a control solution (phosphate-buffered saline). Each discrimination task was comprised of 11 trials, and each trial contained three urine samples from the same donor: two identical 'Lure' samples and one 'Target' sample. Humans were instructed to choose which one of the three samples differed from the other two. Thus, when attempting to identify the post-injection LPS urine from among the two identical pre-treatment urines (or the post-injection control sample from among two identical pre-treatment urines), the rate of correct identification by chance was 33%. The order of LPS and Control discrimination tasks was counter-balanced across human biosensors.

Human biosensors discriminated post- from pre-injection urine samples of donors injected with LPS significantly more often than could be expected by chance ($x=4.45$, $n=20$, $p<0.05$) (*Fig. 3*). Human biosensors also discriminated post- from pre-injection urine samples of control donors ($x=5.75$, $n=20$, $p<0.001$; Figure 2). However, discrimination performances with LPS and PBS post-injection Target samples were not correlated ($n=20$, $r=-0.069$), as tested with the Pearson's correlation.

These results are consistent with the hypothesis that LPS injection alters the changes in human urine odors normally seen over the course of the day. Chemical studies provide stronger support for this hypothesis, as described below.

Chromatographic data from headspace GC/MS analyses (Experiment 6) of the same human urine were subjected to linear discriminant analysis. Using five peaks, 16 of 19 samples collected from LPS-injected Donors and 10 of 13 samples collected from PBS-injected Donors were correctly categorized. The cross-validation error rate was 19.4%.

Together, these results suggest that LPS injection alters the changes in human urine volatiles normally seen over the course of the day in a predictable manner. Differences in human urine odor before and after lipopolysaccharide (LPS) injection are recognizable to human subjects, and can be characterized by changes to five chemical components.

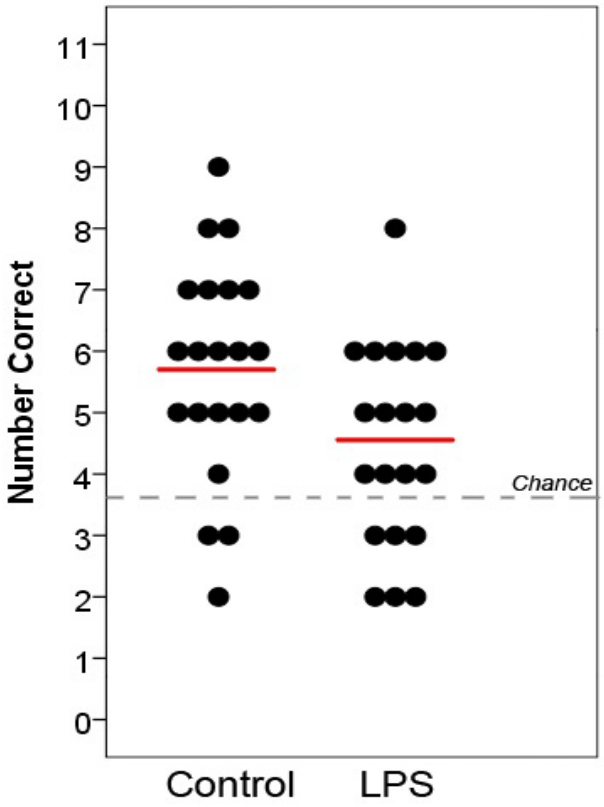

Figure 2. Human subject performance in LPS and control discrimination tasks, as measured by number of correct trials. All subjects (n=20) performed both tasks, and group means are indicated by red lines. Discrimination performances in both the LPS (x=4.45 correct trials) and control (x=5.75) conditions were significantly better than chance performance ($p<0.05$).

Milestone 2. Investigation of the time course of LPS-induced odor change in the mouse model is on schedule and due to be fully completed in Y2Q1. (This milestone will require the completion of Experiment 1.) Chemometric evaluation was completed in Y1Q4; while mouse biosensor evaluation is near completion.

Chromatographic data from 514 urine samples were subjected to principal components analysis. Principle components were modeled versus day of urine collection (post-treatment). When fitted to logarithmic functions, results demonstrate that urine volatile differences between the LPS and control treatment persist for approximately 35 days (Figure 3).

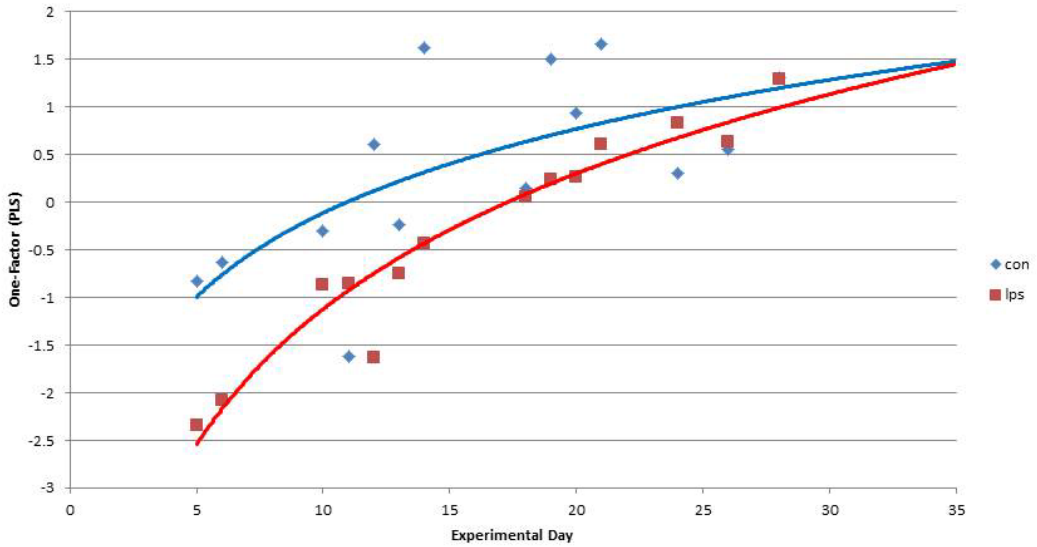

Figure 3. Mouse urine volatile differences between the LPS and control treatment persist for approximately 35 days.

Milestone 3. Investigation of altered body odors in the mouse model of traumatic brain injury (TBI) using both animal biosensors and chemometric analyses is on schedule and due to be completed in Y2Q2. (This milestone will require the completion of Experiments 3 and 4.)

4. KEY RESEARCH ACCOMPLISHMENTS:

- Demonstration that lipopolysaccharide (LPS) induces detectable changes in body odor as evidenced in mouse urine.
- Demonstration that LPS-induced changes to mouse urine odor can be explained by a model using just 3 peaks (chromatographic responses from three individual volatile chemicals).
- Demonstration that LPS induces predictable changes in human urine odor that can be detected by other humans.
- Demonstration that these LPS-induced changes to human urine odor can be explained by a model using only five peaks (chromatographic responses from five individual volatile chemicals).

5. CONCLUSION:

We conclude that LPS-induced inflammation alters urine volatiles in both mice and humans. Furthermore, these changes can be detected by olfaction. Future work will focus on patterns of volatiles associated with traumatic brain injury (TBI) and determine if there are similarities between inflammation caused by LPS and TBI.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

a. Publications

(1) Lay Press:	Nothing to report.
(2) Peer-Reviewed Scientific Journals:	Nothing to report.
(3) Invited Articles:	Nothing to report.
(4) Abstracts:	Nothing to report.

b. Presentations (* indicates associated manuscript)

Gary K. Beauchamp, "Odor Signatures of Inflammation." Annual Meeting of Monell Sponsors, Philadelphia, PA. 08 October 2013.

7. INVENTIONS, PATENTS AND LICENSES: Nothing to report.

8. REPORTABLE OUTCOMES: Nothing to report.

9. OTHER ACHIEVEMENTS: Nothing to report.

10. REFERENCES:

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